L-NAME-INDUCED HYPERTENSION IN THE WKY AND BHR RAT

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Master of Arts

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by

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The development of hypertension in normotensive Wistar-Kyoto (WKY) and borderline hypertensive rats (BHR) was studied by inhibiting the synthesis of nitric oxide, an endothelial-derived relaxing factor. The BHR is genetically predisposed to developing hypertension in response to chronic exposure to environmental stress. After normalizing for initial systolic blood pressure (SBP), rats received drinking water that contained N^{ω}-nitro-L-arginine methyl ester (L-NAME) at concentrations of 0, 75, and 150 mg/L for three to four weeks. SBP, heart rate (HR), body weight (BW), and water consumption were measured weekly throughout L-NAME administration as well as during the subsequent two to three weeks following L-NAME withdrawal. L-NAME at either concentration produced progressively increasing hypertension in both the WKY and BHR rats by 21 days. The L-NAME-induced increase in SBP was reversed in the WKY when L-NAME was withdrawn but not in the BHR. Oral administration of L-NAME had no consistent effect on HR, BW, or water consumption although the administration of the 150 mg/L concentration was in some instances fatal to BHRs. The irreversibility of the L-NAME-induced hypertension in the BHR illustrates the significance of genetic predisposition to the development of sustained hypertension. This method for induction of sustained hypertension in rats (BHR), which can be compared with normotensive litter mates, provides a useful hypertensive model.

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ABBREVIATIONS

ACh	Acetylcholine
ANS	Autonomic Nervous System
BHR	Borderline Hypertensive Rat
BP	Blood Pressure
BPM	Beats Per Minute
BW	Body Weight
CNS	Central Nervous System
EDCF	Endothelial-derived Contracting Factor
EDRF	Endothelial-derived Relaxing Factor
eNOS	Endothelial Nitric Oxide Synthase
ET-1	Endothelin-1
HR	Heart Rate
iNOS	Inducible Nitric Oxide Synthase
L-NAME	N^{ω}-nitro-L-arginine methyl ester
L-NIO	N-iminoethyl-L-ornithine
L-NMMA	N^{ω}–monomethyl-L-arginine
n	Sample Size
NE	Norepinephrine
nNOS	Neuronal Nitric Oxide Synthase
NO	Nitric Oxide
NOS	Nitric Oxide Synthase
PRA	Plasma Renin Activity
SBP	Systolic Blood Pressure
SEM	Standard Error of the Mean
SHR	Spontaneously Hypertensive Rats
VSM	Vascular Smooth Muscle
WKY	Wistar-Kyoto

INTRODUCTION AND REVIEW OF THE LITERATURE

The discovery of vascular endothelial secretory activity opened many new areas in hypertension research. The endothelium is a single-cell layer that lines all arteries and veins. It contributes to the tone of vascular smooth muscle (VSM) and mediates vasodilation in response to various stimuli including shear stress and the neurotransmitter, acetylcholine (ACh) (Furchgott and Zawadzki, 1980). ACh binds to muscarinic cholinergic receptors located in the endothelial cell membrane which initiates the synthesis and release of the autocoid, nitric oxide (NO). Since cell membranes are permeable to small, gaseous molecules, NO readily diffuses into the adjacent VSM cells resulting in relaxation. This relaxation is achieved through the cGMP second messenger system that leads to activation of calcium pumps embedded in the plasma membrane and sarcoplasmic reticulum. The calcium pumps effectively lower the intracellular calcium concentration causing relaxation of VSM and dilation of the blood vessels (review by Shepard and Katusic, 1991).

NO is an example of an endothelial-derived relaxing factor (EDRF). EDRFs are agents produced by the endothelium that stimulate VSM relaxation and subsequent dilation of the blood vessel. Landmark investigations (Ignarro et al., 1987) showed that NO is the primary EDRF regulating VSM. The endothelium also produces certain endothelial-derived contracting factors (EDCF) such as angiotensin II, endothelin-1 (ET-1), prostaglandin H_2, and thromboxane A_2. EDCFs act as vasoconstrictors, that is, they promote VSM contraction (review by Shepard and Katusic, 1991). The collective interaction of

these endothelial-derived factors on VSM contributes toward the regulation of blood vessel tone. There is also an endothelial-derived hyperpolarizing factor which is still unidentified.

NO is produced in the body by endothelial cells as well as by activated macrophages (Stuehr et al., 1989) and neutrophils (Wright et al., 1989). NO is synthesized by the enzyme, nitric oxide synthase (NOS), through the deamination of the amino acid, L-arginine (Forstermann et al., 1991). Currently, three different isoforms of NOS have been identified and studied in mammalian tissue (Forstermann et al., 1993; Nathan and Xie, 1994; Sessa, 1994). The isoforms contain unique amino acid sequences and their activities are influenced by the availability of cofactors. Ca^{2+}, ATP, FAD, FMN, heme, NADPH, O_2, and tetrahydrobiopterin serve as cofactors necessary for NOS function. Endothelial NOS (eNOS) is associated with transient EDRF synthesis (Janssens et al., 1992; Lamas et al., 1992; Nishida et al., 1992; Sessa et al., 1992) and, therefore, is of particular relevance in this study. Calmodulin constitutes a regulatory subunit of the membrane bound eNOS enzyme complex which binds Ca^{2+} in the cytosol of the endothelial cell. eNOS is active at high intracellular Ca^{2+} levels.

Other isoforms of NOS include neuronal NOS (nNOS) and inducible NOS (iNOS). nNOS is involved with signal transduction in central (Garthwaite et al., 1988) and peripheral neurons (Li and Rand, 1989) including those associated with the cardiovascular regulatory center (Forstermann et al., 1990) and perivascular nerves (Toda et al., 1993). nNOS is similar to eNOS, in that it utilizes calmodulin (Bredt et al., 1991) and is involved with transient, Ca^{2+}-dependent, NO

production. iNOS, the third isoform of NOS, contains calmodulin (Cho et al., 1992) but is Ca^{2+}-independent since it is active at normal intracellular Ca^{2+} levels. iNOS was first isolated from macrophages and can be located in most nucleated cells as part of the immune response to pathogens such as viruses (Karupiah et al., 1993; Nathan and Hibbs, 1991). iNOS, unlike eNOS and nNOS, participates in ongoing NO synthesis.

After NO is synthesized, this small, hydrophobic molecule diffuses into neighboring cells. Due to its short half-life (5 to 10 seconds), it acts only locally because it is converted to nitrates and nitrites by oxygen and water in the extracellular space (Wink et al., 1993). Once NO reaches the VSM cell cytosol, it reacts with iron in the active site of the enzyme guanylyl cyclase (Ignarro et al., 1982). This stimulates the production of the intracellular mediator cGMP which greatly amplifies the signal (review by Lamb and Pugh, 1992).

The endothelium synthesizes NO incorporating the terminal guanidino nitrogen of L-arginine (Palmer et. al., 1988a). Synthesis of NO is an enantiomer-specific reaction and is inhibited *in vitro* by N^{ω}-monomethyl-L-arginine (L-NMMA), but not its D-enantiomer (Palmer et al., 1988b). Other experiments showed NOS is also inhibited by N-iminoethyl-L-ornithine (L-NIO) and N^{ω}-nitro-L-arginine methyl ester (L-NAME) in the adrenal gland (Palacios et al., 1989) and brain (Knowles et al., 1990) (Figures 1 and 2).

eNOS is inhibited by guanidino substituted analogues of L-arginine such as L-NMMA, L-NIO, and L-NAME. These analogues, both *in vivo* and *in vitro*, compete with L-arginine for binding sites

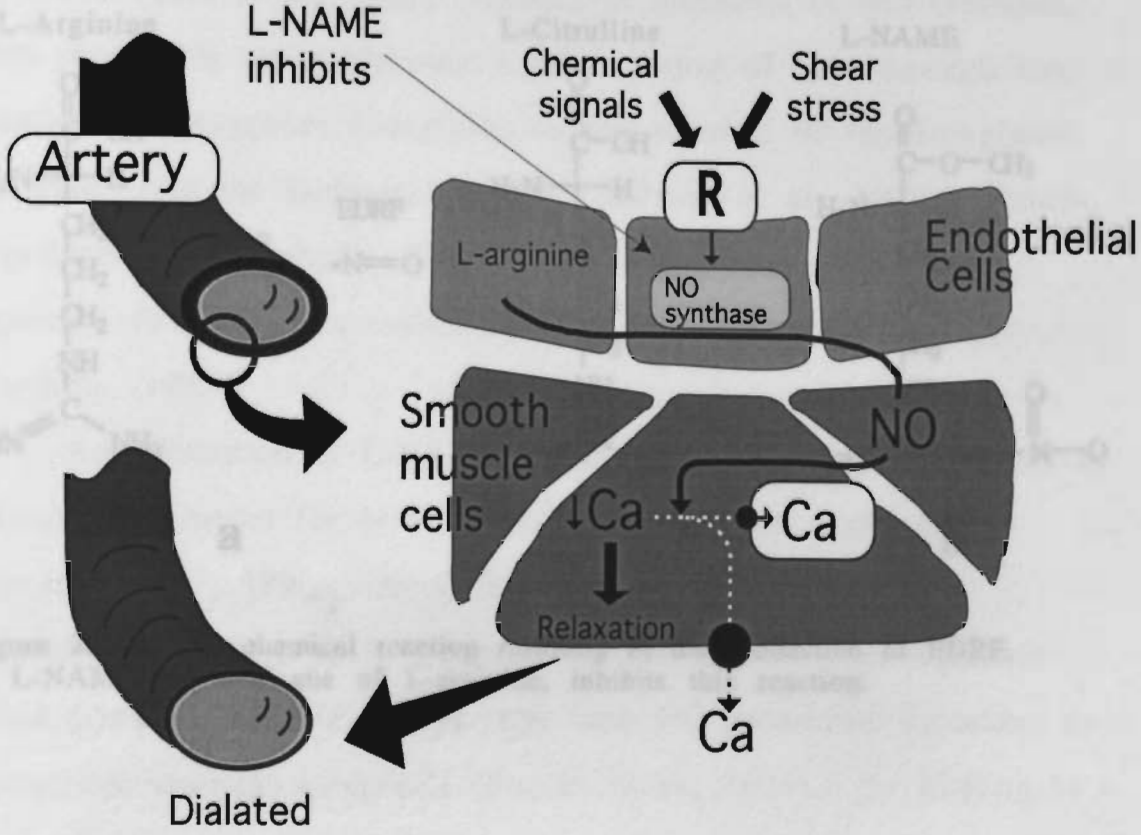

Figure 1. Schematic diagram depicting the synthesis, release, and vasodilatory effect of EDRF.

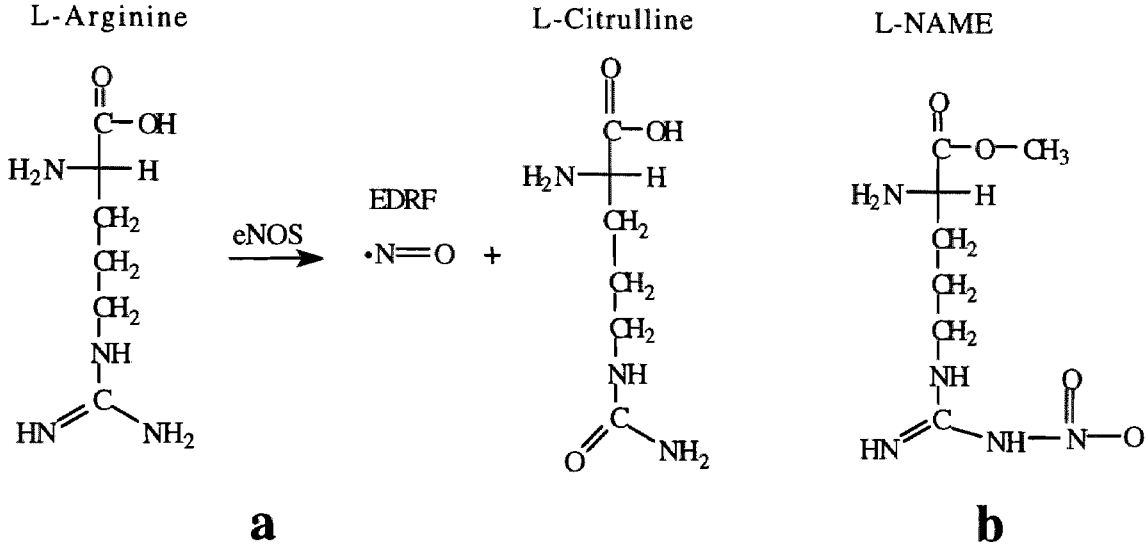

Figure 2. a, The chemical reaction resulting in the production of EDRF. b, L-NAME, an analogue of L-arginine, inhibits this reaction.

on eNOS (Rees et al., 1990), resulting in inhibition of NO synthesis. This process is reversible over a short period of time through the addition of exogenous L-arginine or the removal of the L-arginine analogue from the biological system (Vallance et al., 1989). Recent experiments have shown that increased duration of inhibitor exposure decreases the reversibility of the reaction (Olken and Marletta, 1993).

Administration of L-NAME results in hypertension and serves as an excellent model for *in vivo* study because it is orally active (Gardiner et al., 1990). Aside from the action of L-NAME to competitively inhibit eNOS, other L-NAME actions may influence blood pressure (BP). L-NAME may limit NO production by acting as a muscarinic receptor antagonist (Buxton et al., 1993). By binding to a muscarinic receptor, L-NAME prevents the opening of the receptor operated calcium channel associated with this receptor and effectively eliminates ACh-mediated NO production. This secondary action of L-NAME should be considered in any experiment involving NOS inhibition via L-NAME.

NOS inhibitors have also been shown to decrease plasma renin activity (PRA) in anesthetized (Johnson and Freeman, 1992; Sigmon et al., 1992) and unanesthetized rats (Navarro et al., 1994). In contrast to these studies, other researchers have reported that NOS inhibitors increase PRA (Dananberg et al., 1993; Oliveira et al., 1992; Salazar et al., 1992). Other effects of oral administration of NOS inhibitors include elevated catecholamine (Navarro et al., 1994; Zanchi et al., 1995) and ET-1 (Richard et al., 1995) levels and alterations in prostaglandin activity (Nakaike et al., 1995). These

physiological changes may act synergistically or antagonistically in altering systolic blood pressure (SBP) and, therefore, should be taken into account in any experiment involving L-NAME-induced hypertension.

When studying cardiovascular pathology, it is important to consider all factors involved with the onset of disease. Hypertension can be caused by various factors including stress, high salt intake, genetic predisposition, obesity, or vasoactive drugs. Early experiments with L-NAME-induced hypertension involved normotensive models such as the Brattleboro (Gardiner et al., 1990) and Wistar-Kyoto (WKY) rats (Rees et al., 1990). As stated earlier, this hypertension is reversible over a short period of time when L-NAME is removed. The Borderline Hypertensive Rat (BHR) represents an interesting model for hypertension research (Lawler et al., 1980).

The BHR is the first generation offspring produced by mating female spontaneously hypertensive rats (SHR) with male WKY rats. Unlike the normotensive WKY rat, the BHR is genetically predisposed to developing hypertension. In the BHR, the onset of hypertension can be accelerated by environmental or physiological stimuli such as high salt diet. Further, unlike the WKY rat, the hypertension will persist even after removal of the high salt diet (Lawler et al., 1988) or other stressors (Lawler et al., 1981). By three months of age, male BHRs will have resting SBP measurements of approximately 135 mmHg versus approximately 120 mmHg for the WKY (Morrow and Stratton, 1995). Consequently, one of the questions addressed

by this study is to determine if L-NAME can produce hypertension in the BHR, and if so, will it persist following L-NAME withdrawal?

A survey of the research literature in which L-NAME has been used to induce hypertension provides no consensus on the appropriate concentration of L-NAME for oral administration. Reported concentrations in the drinking water range from 50 mg/L (Navarro et al., 1994) to 500 mg/L (Henrion et al., 1996). The inhibition of NO synthesis over extended periods of time results in premature death (8 to 11 weeks in rats) due to organ damage (Arnal et al., 1992; Blot et al., 1994). This is surprising considering that the SHR can survive with similar SBP for up to 70 weeks (Kung and Luscher, 1995). A review of the literature has failed to demonstrate a consistent relationship between the amount of L-NAME ingested and the degree of eNOS inhibition. Consequently, a second question addressed by this study will be to determine an effective concentration of L-NAME for producing consistent and reliable hypertension.

In summary, this study will examine the effects of NO inhibition on WKY and BHR rats. The subsequent reduced influence of NO on VSM in response to increasing L-NAME concentrations will be evaluated by measuring SBP. The reversibility of L-NAME-induced hypertension will be studied by replacing the L-NAME solution with normal tap water and continuing to monitor concomitant changes in SBP.

MATERIALS AND METHODS

Materials

The Wistar-Kyoto (WKY) rats were purchased from Taconic Farms, Germantown, New York while the Borderline Hypertensive Rats (BHRs) were bred from stock maintained within the Animal Care Facility at Drake University, Des Moines, Iowa. All rats were given at least a two day acclimatization period prior to any experimental manipulations. The rats were housed individually in standard stainless steel cages (with a length of 26 cm, width of 21 cm, and height of 21 cm) and exposed to a twelve hour light/dark cycle. All rats were fed Harlan/Teklad LM-485 sterilizable mouse/rat diet 7012.

The drug used was N^{ω}–nitro-L-arginine methyl ester (L-NAME) which dissolved readily in tap water. It was obtained from Sigma Chemical Company, St. Louis, Missouri. Stocks of 10 L were made on a weekly basis from which the individual water bottles were filled. The final concentration was expressed as mg/L.

L-NAME and WKY rats

After weaning at four weeks of age, 27 male WKY rats were fed standard rat chow and tap water *ad libitum* for a four week period. At eight weeks of age, the body weight (BW) (g), heart rate (HR) (beats/minute (BPM)), and systolic blood pressure (SBP) (mmHg) of the WKY rats were measured. The HR and SBP were measured

indirectly via tail cuff sphygmomanometry (Stratton et al., 1994) utilizing a programmed electro-sphygmomanometer and pneumatic pulse transducer (Narco Bio-Systems, Austin TX). Pfeffer et al. (1971) showed that SBPs determined by this method were consistent with direct blood pressure (BP) recordings of the carotid artery.

To make these measurements, rats were restrained in the small animal study unit, were shielded from excessive visual stimulation, and were heated on a hot strip set at 40°C using the temperature control unit (Narco Bio-Systems, Austin TX). A minimum of 15 minutes of acclimatization was allowed to assure adequate perfusion of the tail and to reduce stress induced changes in BP. An occluding cuff was placed around the base of each rat's tail with the bulb of the pneumatic pulse transducer positioned distal to the cuff to detect pulsatile flow changes in the caudal artery. The cuff was alternately inflated and deflated to 200 mmHg at a rate of 20 mmHg/second. During acclimatization, the cuff was inflated and deflated several times. The sensitivity of the programmed electro-sphygmomanometer was uniformly maintained by setting the pressure amplitude at 4.6 and adjusting the sound-pulse amplitude over a range of 3 to 5. The pressure amplitude control determines the magnitude of graphical excursion in response to changes in the occluding cuff pressure (e.g. 100 mmHg = 3 cm of graphical excursion). The sound-pulse amplitude control regulates the sensitivity of the pneumatic pulse transducer. Pulsatile changes were recorded using the MacLab™ computer-aided data acquisition system running Chart software v.3.2 (AD Instruments, Castle Hill, NSW 2154, Australia) linked to a Macintosh IIci computer (Figure 3).

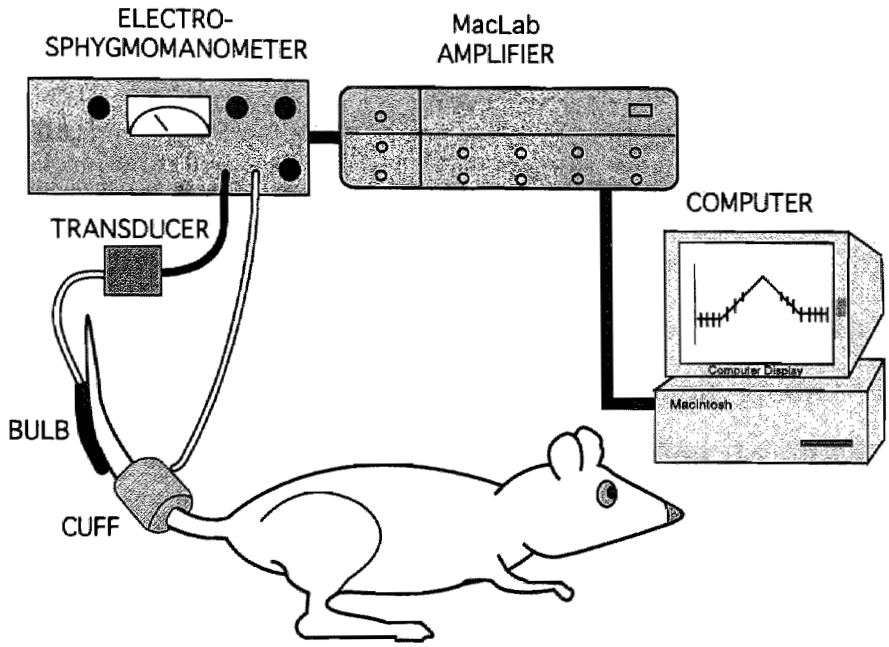

Figure 3. The apparatus used for measuring SBP.

The numerical average of three clearly measurable traces was recorded as each rat's SBP. This apparatus also measured HR and experimental values were obtained either before or after SBP determinations.

After recording these measurements at eight weeks of age, the WKY rats were divided into three treatment groups matched by the initial SBP. This procedure involved arranging rats by magnitude of initial SBP and allocating every third rat to a different group. The order of allocation was reversed after placing the first 14 WKY rats in treatment groups to prevent the assignment of all rats with relatively low or high SBPs to the same group. All were fed standard rat chow *ad libitum*. Each group was allowed free access to tap water and the experimental groups received one of two different concentrations of L-NAME (75 mg/L or 150 mg/L) for the next four week period. L-NAME was then withdrawn and all WKY rats received tap water with no L-NAME for an additional two weeks.

The water consumption of each animal was monitored throughout the experiment and was expressed as milliliters of water consumed per day and as milliliters of water consumed per 100 grams of BW. The amount of drug ingested was calculated and was expressed as milligrams of L-NAME per kilogram of BW. BW, HR, and SBP were measured weekly at approximately the same time (0700 to 1700). All rats were studied in the same order throughout the experiment and treatment groups were amalgamated to eliminate diurnal influences. Table 1 summarizes the entire protocol involving the WKY rats.

Table 1. Chart summarizing the experimental procedure for the WKY and BHR rats. X indicates age of rat in weeks at time of experimental manipulation.

Procedure	Age in Weeks										
	4	5	6	7	8	9	10	11	12	13	14
Wean **WKY** and **BHR** s	X										
House **WKY** and **BHR** s Individually		X	X	X	X	X	X	X	X	X	X
Monitor **WKY** water consumption					X	X	X	X	X	X	X
Record SBP, HR, and BW of **WKY** and **BHR** s					X	X	X	X	X	X	X
Match **WKY** and **BHR** s by initial SBP					X						
Add L-NAME to water of **WKY** rats						X	X	X	X		
Add L-NAME to water of **BHR** s						X	X	X			
Remove L-NAME from water of **WKY** rats										X	X
Remove L-NAME from water of **BHR** s									X	X	X

L-NAME and BHRs

A similar protocol was followed using 16 male BHRs. At approximately eight weeks of age, they were divided into two treatment groups matched by the initial SBP. The BHRs were housed individually and fed standard rat chow *ad libitum* throughout the experiment. One treatment group was administered 150 mg/L of L-NAME dissolved in tap water for three weeks while the other treatment group received drug free tap water. For three additional weeks, all BHRs received tap water with no drug. BW, HR, and SBP were measured weekly throughout the experiment. Table 1 summarizes the entire protocol involving the BHRs.

Statistical Analysis

The weekly values for each experimental group were plotted as the mean $\pm$ standard error of the mean (SEM). The means were analyzed using ANOVA followed by Bonferroni's correction for multiple comparisons. Analyses were performed and graphs were designed using Abacus Concepts, StatView (Abacus Concepts, Inc., Berkeley, CA, 1992).

RESULTS

L-NAME and WKY Rats

Systolic Blood Pressure of WKY Rats in Response to 75 mg/L and 150 mg/L Concentrations of L-NAME in the Drinking Water.

The Wistar-Kyoto (WKY) rats that ingested either concentration of N^{ω}–nitro-L-arginine methyl ester (L-NAME) showed significantly elevated systolic blood pressure (SBP) measurements after 14 days when compared to the control group (Figures 4 and 13, Table 2). Their SBP measurements continued to increase substantially the next two weeks with the highest SBP recordings for the group administered 150 mg/L of L-NAME. Statistically significant differences in SBP were observed between the 75 mg/L L-NAME and 150 mg/L L-NAME groups over weeks three and four. The SBP measurements decreased to control levels in the L-NAME treatment groups over the final 14 days coinciding with the reintroduction of drug free tap water. The SBP recordings of the high and low L-NAME concentration groups remained significantly different from SBP readings of the control group during the fifth week despite diminishing SBP readings for both groups. At six weeks, there were no statistically significant differences in SBP.

Body Weight and Water Consumption of WKY Rats in Response to 75 mg/L and 150 mg/L Concentrations of L-NAME in the Drinking Water.

The body weights (BWs) of the WKY rats increased comparably in all three treatment groups irrespective of the presence or absence of L-NAME (Figure 5, Table 2). All three groups exhibited a similar growth pattern with mean BWs approximating the same values throughout the experiment. Over the 42 day period, the mean BW rose from 280 grams to 455 grams with an average daily gain of 10.8 grams/day. Accompanying this normal rate of growth, water consumption increased by 8 ml/day regardless of the concentration of L-NAME in the tap water (Figure 6). The administration of L-NAME via the tap water did not significantly alter water consumption in the WKY rats receiving L-NAME when compared to the control group. Water consumption decreased, however, on a milliliters of water consumed per 100 grams of BW basis as the animals matured (Figure 7). Further, the water consumption measurements indicate that the WKY rats administered 150 mg/L of L-NAME consistently received twice as much drug (16.9 mg L-NAME/ kg BW to 8.24 mg L-NAME/ kg BW) when compared to the WKY rats administered 75 mg/L of L-NAME (Figure 8).

Heart Rate of WKY Rats in Response to 75 mg/L and 150 mg/L Concentrations of L-NAME in the Drinking Water.

None of the treatment groups exhibited a consistent pattern of heart rate (HR) alteration (Figure 9). The HRs fluctuated over a

broad range of values (329 beats/minute (BPM) to 376 BPM) throughout the experiment. During the sixth week, however, the WKY rats subjected to the high dose of L-NAME demonstrated a significant increase in HR. No other significant variations in HR were observed.

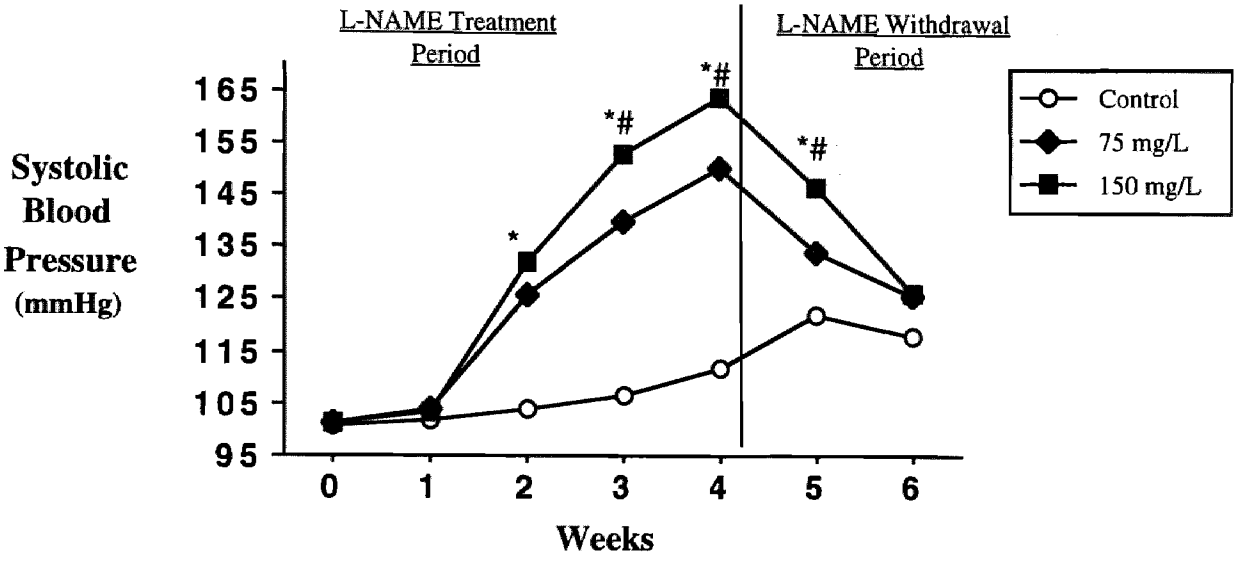

Figure 4. The SBP measurements of the WKY rats in response to two different concentrations of L-NAME. Control rats received no L-NAME. *($p<0.0167$) 150 mg/L and 75 mg/L different from control. #($p<0.0167$) 150 mg/L different from 75 mg/L.

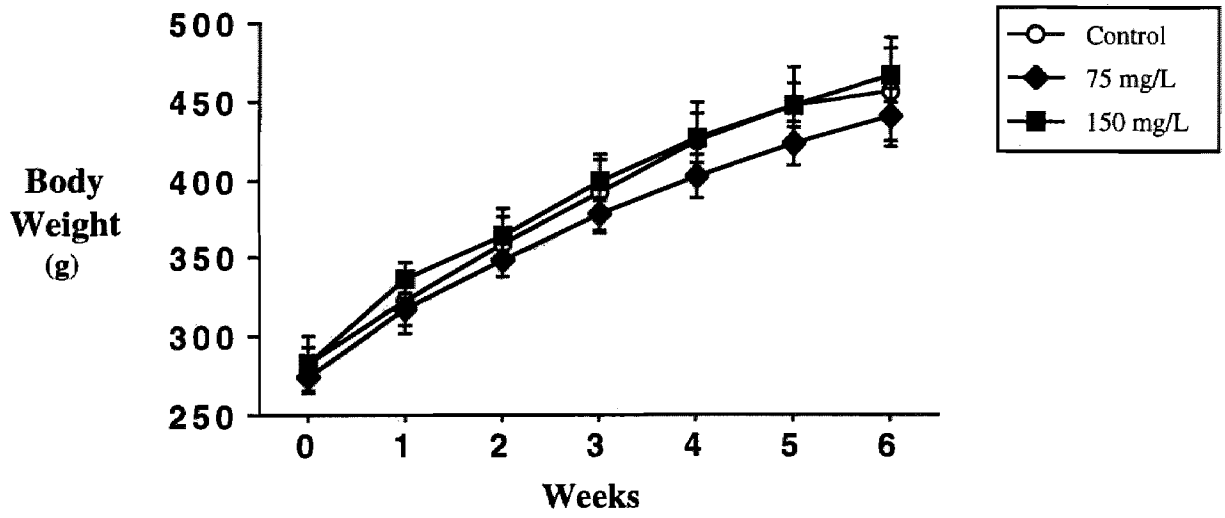

Figure 5. The BWs of the WKY rats in response to two different concentrations of L-NAME.

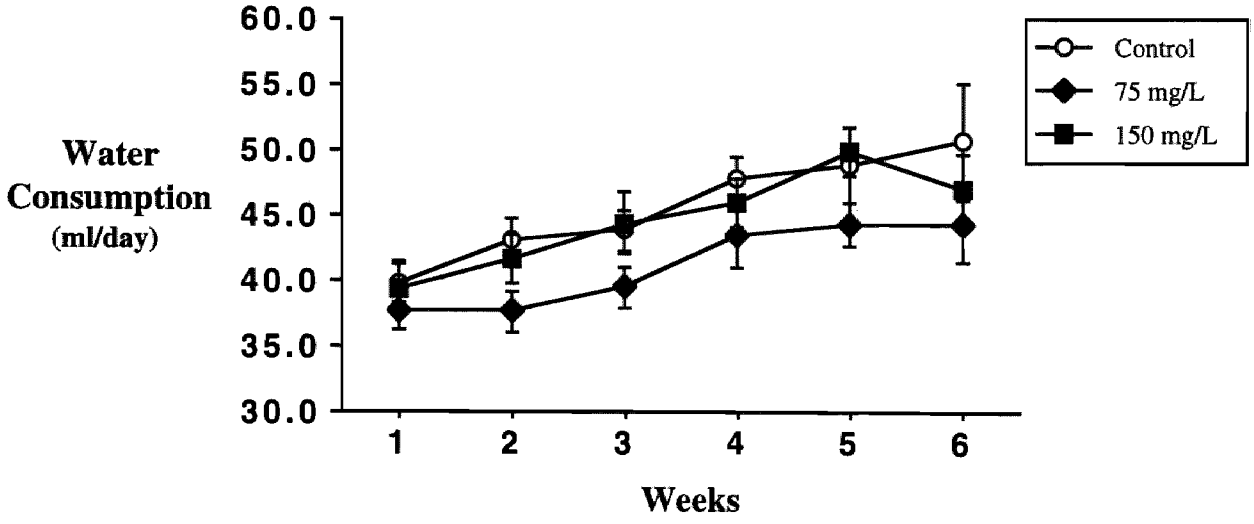

Figure 6. The water consumption of the WKY rats in response to two different concentrations of L-NAME.

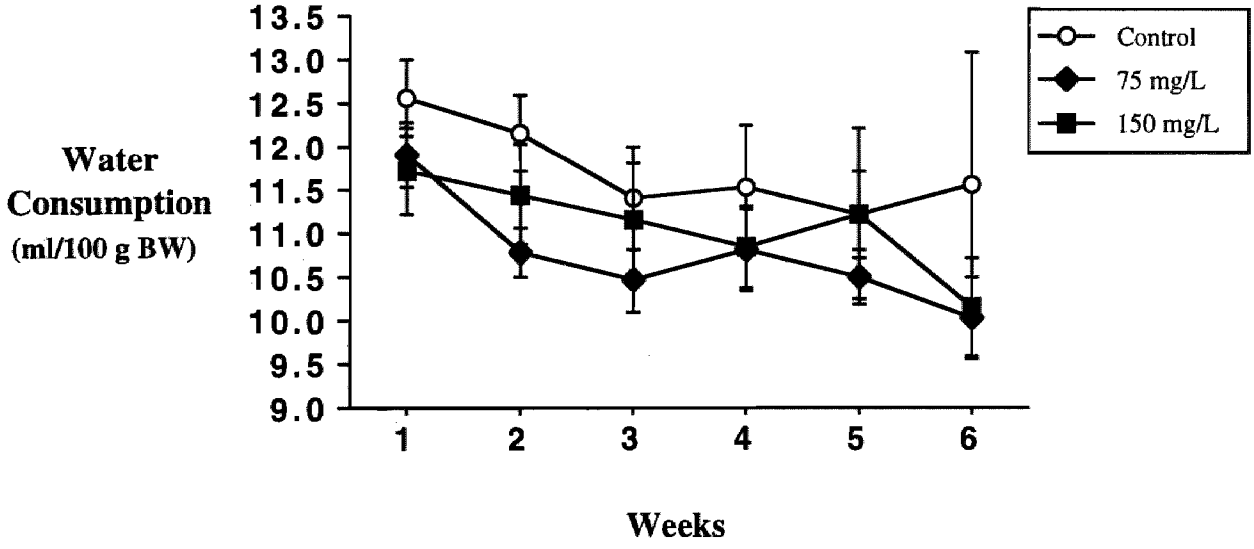

Figure 7. The water consumption of the WKY rats in response to two different concentrations of L-NAME as expressed per 100 grams of BW.

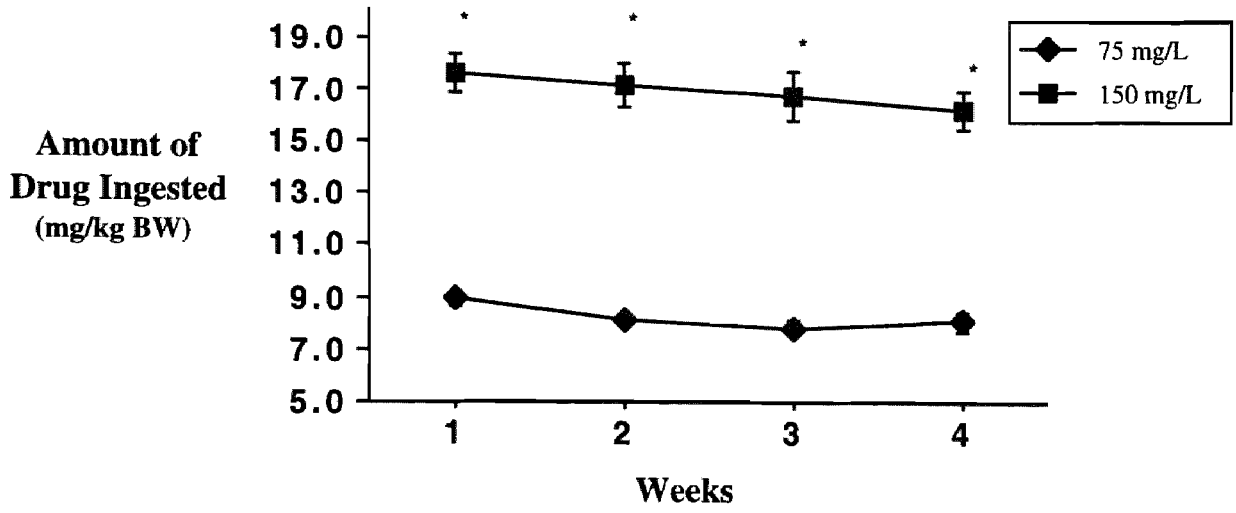

Figure 8. The concentration of L-NAME administered to the WKY rats as expressed per kilogram of BW. *($p<0.0001$) 150 mg/L different from 75 mg/L.

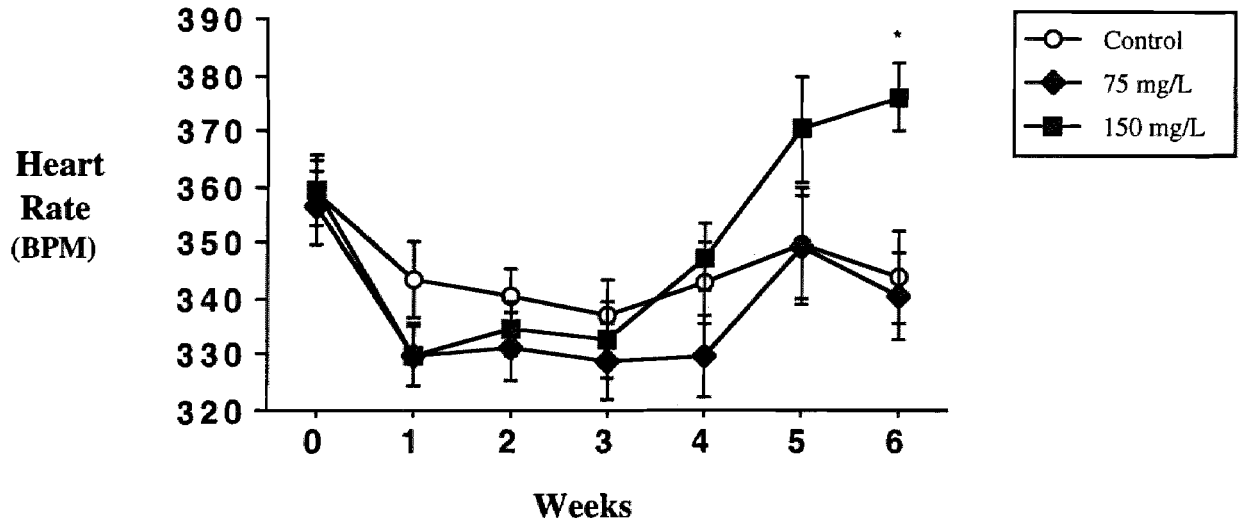

Figure 9. The HRs of the WKY rats in response to two different concentrations of L-NAME. *($p<0.0167$) 150 mg/L different from control and 75 mg/L.

L-NAME and BHRs

Systolic Blood Pressure of BHRs in Response to 150 mg/L Concentration of L-NAME in the Drinking Water.

The SBP measurements of the Borderline Hypertensive Rats (BHRs) administered L-NAME were significantly higher than those of the control group throughout the experiment (Figures 10 and 13, Table 2). The BHRs receiving L-NAME exhibited marked hypertension after 21 days and remained hypertensive even after the withdrawal of L-NAME. Mean SBP measurements approximated 170 mmHg after 28 days and subsequent measurements resulted in similar mean values during the withdrawal period. The SBP measurements of the control group increased gradually over the 42 day period but values remained normotensive.

One BHR administered 150 mg/L of L-NAME expired after 21 days of treatment. Five BHRs were euthanized during the ensuing six days after exhibiting the following symptoms: lethargy, suppressed appetite, and loss of motor function in the pelvic appendages. Therefore, the sample size (n) was reduced to two over the final two weeks of the experiment for the affected treatment group.

Body Weight of the BHRs in Response to 150 mg/L Concentration of L-NAME in the Drinking Water.

The BWs of both treatment groups increased linearly over the first 14 days of the experiment (Figure 11, Table 2) with an average

daily gain of 5.1 grams/day. The BWs of the BHRs receiving L-NAME decreased 33.9 grams during the third week of drug administration. Statistically significant differences in mean BW were recorded after 21 days of L-NAME treatment. These differences remained significant during the last three weeks of the experiment despite evidence of compensatory weight gain after L-NAME withdrawal.

Heart Rate of BHRs in Response to 150 mg/L Concentration of L-NAME in the Drinking Water.

The mean HR of the control group was 376 BPM with minimal deviation from this value over the 42 day period (Figure 12). The BHRs administered 150 mg/L of L-NAME showed no consistent pattern of HR alteration. HRs fluctuated over a wide range of values (331 BPM to 433 BPM) throughout the experiment. Significant differences in HR between the two treatment groups were recorded after seven and 28 days.

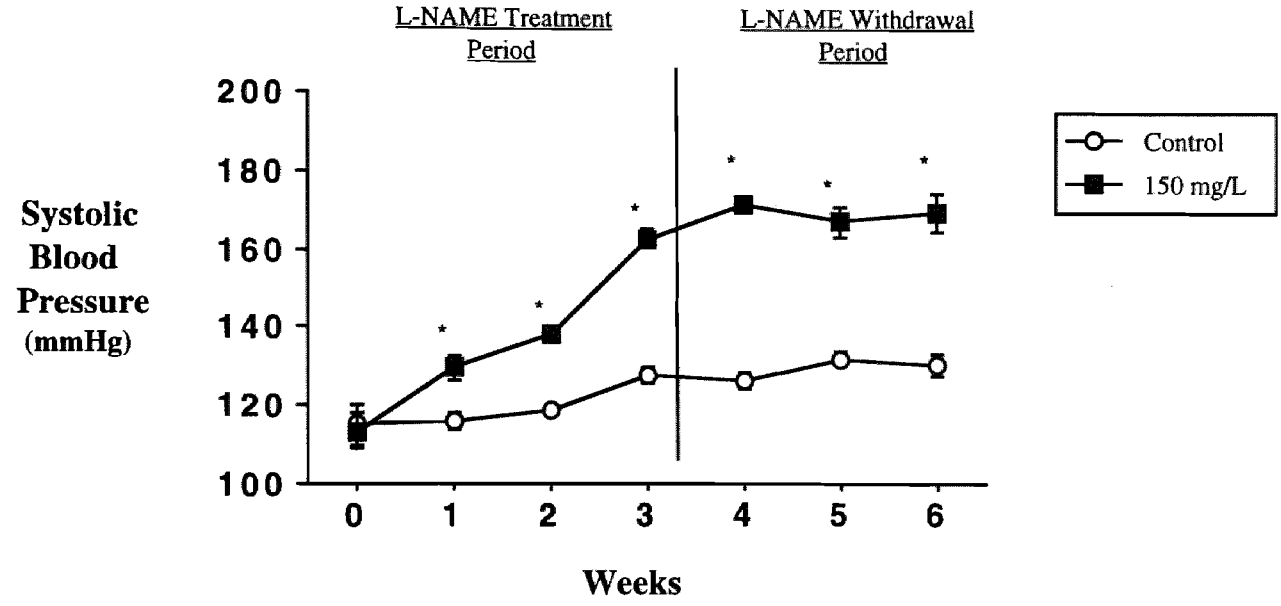

Figure 10. The SBP measurements of the BHRs in response to L-NAME. Control rats received no L-NAME. *($p < 0.0024$) 150 mg/L different from control.

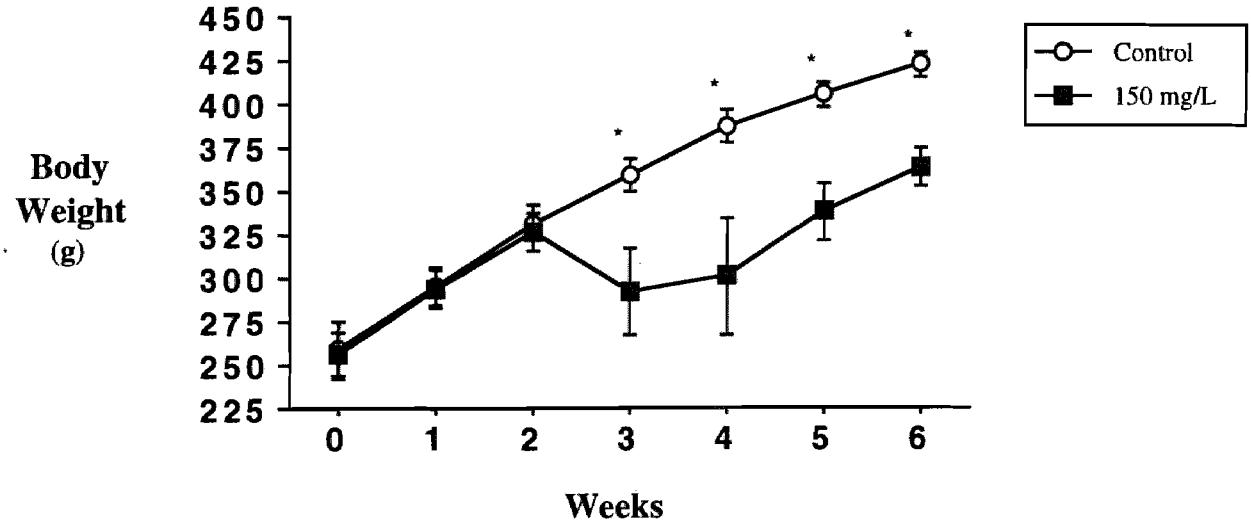

Figure 11. The BWs of the BHRs in response to L-NAME. *($p<0.0262$) 150 mg/L different from the control.

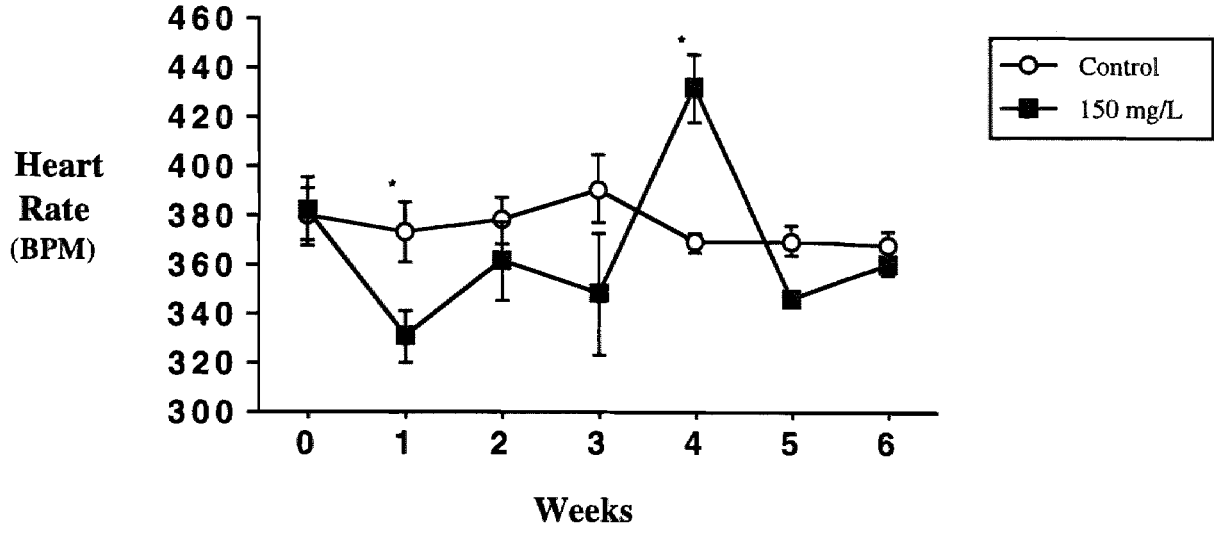

Figure 12. The HRs of the BHRs in response to L-NAME. *(p<0.0203) 150 mg/L different from the control.

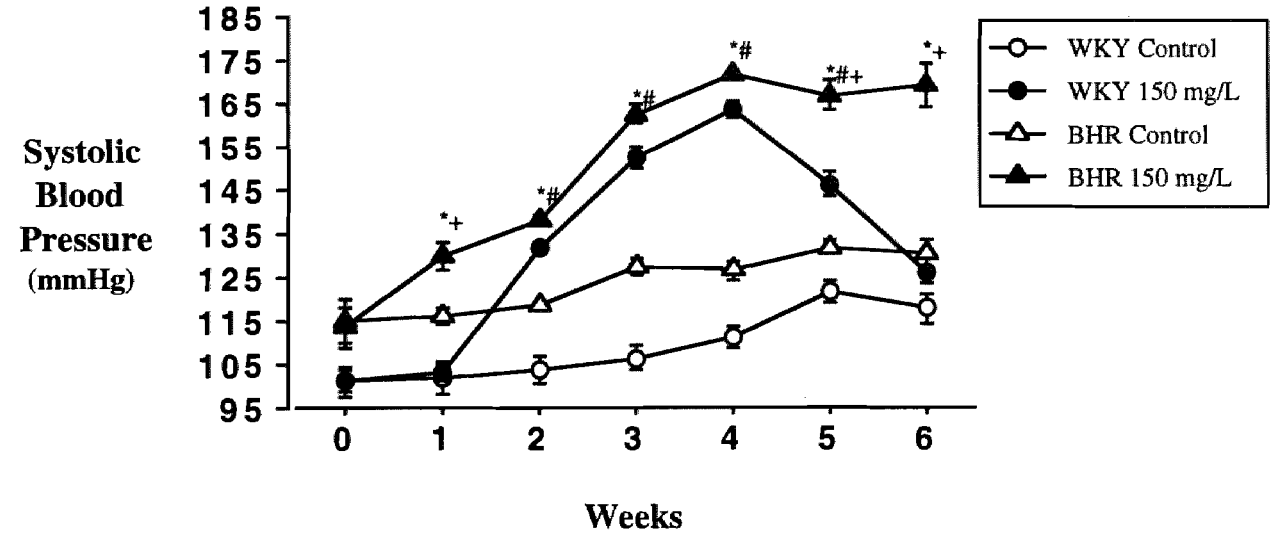

Figure 13. The SBP measurements of the WKY and BHR rats in response to 150 mg/L concentration of L-NAME. Control rats received no L-NAME. *(p<0.0024) BHR 150 mg/L different from BHR control. #(p<0.0167) WKY 150 mg/L different from WKY control. +(p<0.0083) BHR 150 mg/L different from WKY 150 mg/L.

Table 2. The BWs and SBPs of the WKY and BHR rats administered the 150 mg/L concentration of L-NAME. Control rats received no L-NAME. The values are means derived from measurements taken at the start of the experiment (week 0), after three to four weeks of L-NAME administration (week 4), and following the subsequent removal of L-NAME from the tap water (week 6).

	Week 0			Week 4			Week 6		
Group	n	BW	SBP	n	BW	SBP	n	BW	SBP
WKY Control	9	282	101	9	425	111	8	456	118
WKY 150 mg/L	9	282	101	9	427	164*	9	467	126
BHR Control	8	259	115	8	388	127	8	423	130
BHR 150 mg/L	8	256	113	2	301#	172#	2	364#	169#

The sample size (n) described the number of rats per treatment group; BWs were measured in grams; SBP recordings were measured in mmHg. *($p<0.0167$) WKY 150 mg/L different from WKY control. #($p<0.0262$) BHR 150 mg/L different from BHR control.

DISCUSSION

Oral administration of N$^{\omega}$-nitro-L-arginine methyl ester (L-NAME) induces consistent and reliable hypertension in both the Wistar-Kyoto (WKY) and Borderline Hypertensive Rat (BHR) at a concentration of 150 mg/L (Figure 13, Table 2). The observed increases in the systolic blood pressure (SBP) of WKY rats after the oral administration of L-NAME agree with the findings of other studies using similar concentrations of L-NAME (Navarro et al., 1994; Qiu et al., 1994). The value of a BHR model for hypertension is that the blood pressure (BP) remains elevated for several months after an environmental stress is withdrawn. Thus, the hypertensive BHR can be compared to normotensive litter mates long after the hypertensive stimulus is withdrawn. The use of L-NAME constitutes an attractive method for developing hypertension in the BHR in comparison to current, commonly used techniques. A frequently used procedure for elevating SBP in the BHR consists of prolonged exposure to a noxious environmental stimulus such as an electric shock (Lawler et al., 1980; Lawler et al., 1981). Air jet noise is another environmental stimulus used to induce hypertension in the BHR (Fisher and Tucker, 1991). These procedures require extended conditioning periods and are often labor intensive. Other protocols utilize a physiological stimulus such as a high salt diet for hypertension development (Lawler et al., 1988). This protocol introduces the added physiological complications of hypernatremia and natriuresis. L-NAME, however, produces hypertension by acting as a pharmacological stimulus. L-NAME-induced hypertension

simplifies experimental design by providing an alternative to exposing BHRs to noxious environmental or physiological stimuli.

L-NAME elevates SBP through a myriad of physiological mechanisms involved with BP modulation. As a competitive inhibitor of endothelial nitric oxide synthase (eNOS), L-NAME blocks the synthesis of the autocoid nitric oxide (NO), an endothelial-derived relaxing factor (EDRF). The decrease in relaxing factor results in an increased peripheral resistance and a concomitant rise in SBP. This is believed to be the primary mechanism of hypertension employed by L-NAME (Bank et al., 1994). Recent evidence indicates that L-NAME may also act as a muscarinic receptor antagonist further enhancing its effect as a hypertensive agent by competitively inhibiting acetylcholine (ACh)-mediated vasodilation (Buxton et al., 1993). Although not statistically significant, the recorded HRs of the WKY and BHR rats in this study (Figures 9 and 12) revealed a trend toward a drop in HR for all treatment groups except the WKY and BHR controls. The observed decrease in HR may be compensatory and certainly runs counter to any antimuscarinic chemotropic actions due to the oral consumption of L-NAME. This trend continued throughout the period of L-NAME administration in both the WKY and BHR rats until L-NAME withdrawal. The WKY and BHR rats administered 150 mg/L of L-NAME exhibited an increase in HR following the removal of L-NAME from the drinking water.

Since Ignarro et al. (1987) first described NO as the EDRF produced and released from arteries and veins, many additional paracrine functions for NO have been proposed. Consequently, L-NAME, as an orally active agent, may alter other cellular activities regulated by

this ubiquitous chemical messenger. L-NAME competitively inhibits all three isoforms of nitric oxide synthase (NOS) (Knowles et al., 1990; Palacios et al., 1989; Rees et al., 1990). Inhibition of inducible NOS (iNOS) may disrupt the immune response to pathogens in most nucleated mammalian cells including macrophages (Karupiah et al., 1993; Nathan and Hibbs, 1991). iNOS catalyzes the production of NO for subsequent use as a cytotoxin against pathogens. Inhibition of neuronal NOS (nNOS) may prevent NO from acting as a signaling molecule in the brain (Garthwaite et al. 1988) or as a neurotransmitter in peripheral nerves (Li and Rand, 1989). These findings suggest that L-NAME-induced hypertension could involve alterations in the BP regulating activities of the central nervous system (CNS) and autonomic nervous system (ANS). The existence of nNOS activity within the medulla oblongata (Forstermann et al., 1990) potentially implicates an additional hypertensive effect of L-NAME involving nNOS inhibition within the cardiovascular regulatory center. Toda et al. (1993) postulates that L-NAME inhibits nitroxidergic nerve activity by inhibiting the production of NO. "Nitroxidergic" is a term used to describe nerves that use NO as a neurotransmitter in signal transduction. Many of these nitroxidergic nerves are perivascular and cause vasodilation of blood vessels upon activation (Toda and Okamura, 1991). The significance of nNOS inhibition with respect to VSM tone requires further investigation.

L-NAME further influences SBP by regulating plasma renin activity (PRA). High levels of cGMP in juxtaglomerular cells result in the inhibition of renin release. As described previously, NO stimulates cGMP production by activating guanylyl cyclase.

Therefore, inhibition of NO synthesis enhances renin release (Zanchi et al., 1995) by decreasing cGMP levels in juxtaglomerular cells (Sigmon et al., 1992). Navarro et al. (1994) have postulated that limiting NO production through the administration of L-NAME, for example, may actually attenuate pressure-dependent renin release. Under normal physiological conditions, elevations in blood pressure lead to reductions in PRA. Further, NO may be the paracrine mediator that inversely links renal perfusion pressure with the stimulation of renin release (Knoblich et al., 1996). Based on these two observations, NOS inhibition would lead to decreased renin release. Low circulating renin levels lower SBP by lessening the vasoconstrictive activity of the renin-angiotensin system. These physiological mechanisms would actually ameliorate the L-NAME-induced hypertension.

An additional mechanism of L-NAME-induced hypertension may involve elevations in circulating catecholamines. Oral administration of L-NAME increased plasma levels of epinephrine and norepinephrine (NE) in rats (Navarro et al., 1994; Zanchi et al., 1995). Of particular interest was the finding by Navarro et al. (1994) that only rats receiving a high concentration of L-NAME (300 mg/L) exhibited marked elevations in circulating catecholamines. This was contrary to other experimental groups receiving lower concentrations of L-NAME. By raising catecholamine levels, L-NAME indirectly enhances its hypertensive effect via stimulation of alpha adrenergic receptors.

In this study, L-NAME-induced hypertension was reversible in the WKY rat when animals received water without L-NAME (Figure

13 and Table 2). Thus, the hypertensive activity of L-NAME was transient and SBP measurements returned to basal levels within 14 days. In contrast, the L-NAME-induced hypertension was irreversible in the BHR even after L-NAME withdrawal. These results emphasize the significance of genetic predisposition in the development of sustained hypertension.

Folkow (1982) hypothesized that the susceptibility of an organism to developing hypertension in response to an environmental stressor is determined by the organism's genetic bias to high arterial pressure. This genetic bias potentially affects a multitude of BP regulating mechanisms but published reports indicate that CNS, kidneys, and ANS play critical roles (review by Sanders and Lawler, 1992). The amygdala, hypothalamus, pons, and medulla oblongata contain high densities of alpha$_2$ adrenergic receptors (Moore and Bloom, 1979). Alpha$_2$ adrenergic agonists suppress the activation of the ANS (Charney et al., 1983). An impairment in alpha$_2$ adrenergic receptors in the BHR may result in an overstimulation of the ANS and a concomitant hypersecretion of catecholamines from the adrenal medulla in response to an acute stressor (Hubbard et al., 1986b). This impairment may involve altering ligand binding to alpha$_2$ receptors in the CNS. Lundin and Thoren (1982) described the antinatriuretic effect air jet exposure had on SHRs but not WKY rats and, therefore, implicated that the genetic predisposition of the BHR towards hypertension may involve alterations in renal function. Exaggerated circulating plasma NE levels have been demonstrated in the BHR in response to tail-shock stress thus involving the ANS in the onset of hypertension (Hubbard et al., 1986a). All of these

physiological responses increase SBP and may predispose the BHR to developing hypertension.

Future experimentation with BHRs should employ lower concentrations of L-NAME. L-NAME induced concentration-dependent hypertension in the WKY rats as indicated by Figure 4. The concentrations of L-NAME used in this study (75 and 150 mg/L) were similar to those used by Gardiner et al. (1990) (100 mg/L) when L-NAME was first described as being orally active. Findings in this study indicate that administering higher levels of L-NAME causes a malignant form of hypertension. BHRs receiving 150 mg/L of L-NAME may exhibit decreased BW, lethargy, and stroke as evidenced by loss of motor function. Counter to these observations, Zanchi et al. (1995) induced hypertension in male Wistar rats by administering 400 mg/L of L-NAME over a six week period and only observed a small decrease in BW (without any reported evidence of stroke). A concentration of 75 mg/L, while still provoking significant elevations in SBP in WKY rats, may decrease the incidence of stroke observed in BHRs administered 150 mg/L of L-NAME.

By using a lower concentration of L-NAME, it may be possible to determine the specific mechanisms of action of L-NAME in elevating SBP (besides eNOS inhibition). One possible approach would involve contracting blood vessel rings from the superior mesenteric artery (as a model for resistance blood vessels) in a defined medium containing L-NAME in a physiological concentration similar to levels achieved during oral administration. Adrenergic agonists would be of special interest since the ANS appears to play a major role in

L-NAME-induced hypertension (review by Sanders and Lawler, 1992).

Experimental protocols with a prolonged period of L-NAME withdrawal may prove advantageous. This study demonstrated that L-NAME-induced hypertension in the BHR is not reversed by removal of L-NAME from the tap water. This observation was based on three weeks of SBP measurements following L-NAME withdrawal and does not necessarily predict the long term effect of L-NAME on sustained hypertension in the BHR.

Oral administration of L-NAME to the BHR serves as a useful model for studying sustained hypertension. Physicians and researchers have long suspected that genetic predisposition plays a key role in the onset of primary hypertension. Experimentation with the BHR allows researchers to focus efforts on the genetic component of hypertension and further delineate the underlying mechanisms of vascular pathology.

CONCLUSION

This study demonstrates that oral administration of N^{ω}-nitro-L-arginine methyl ester (L-NAME) via tap water can induce hypertension in the Wistar-Kyoto (WKY) rat at concentrations of 75 and 150 mg/L and in the Borderline Hypertensive Rat (BHR) at a concentration of 150 mg/L. L-NAME-induced hypertension is reversible in the WKY but not the BHR rat when L-NAME is withdrawn from the tap water illustrating the significance of genetic predisposition in the development of sustained hypertension. The 75 mg/L concentration can produce significant hypertension in three weeks without additional pathological complications. Since L-NAME is orally active, this technique is not labor intensive. This method of induction of sustained hypertension in rats (BHR), which can be compared with normotensive litter mates, provides a useful hypertensive model.

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